Journal of Elementology ISSN 1644-2296

Ertekin, E., Bilgen, M. and Ertekin, I. (2025)
'Evaluation of germination dynamics and seedling vigor under varying heavy metal concentrations in key legume forage plants',
Journal of Elementology, 30(4), 863-884,
available: https://doi.org/10.5601/jelem.2025.30.3.3632

RECEIVED: 4 September 2025
ACCEPTED: 2 November 2025

ORIGINAL PAPER

Evaluation of germination dynamics and seedling vigor under varying heavy metal concentrations in key legume forage plants*

Esra Nermin Ertekin[1], Mehmet Bilgen[2], Ibrahim Ertekin[1]

[1] Department of Field Crops
Hatay Mustafa Kemal University, Hatay, Türkiye
[2] Department of Field Crops
Akdeniz University, Antalya, Türkiye

Abstract

Heavy metal contamination poses significant risks to plant germination and early seedling development. This study investigated the effects of different heavy metal types (Cd, Hg, Cr, Pb, Ni), concentrations (0, 100, 200, 400, 800 mg L^{-1}), and plant species (sainfoin, common vetch, and alfalfa) on seed germination parameters and seedling growth traits. The experiment was conducted under controlled laboratory conditions at the Department of Field Crops, Faculty of Agriculture, Akdeniz University. The results revealed that heavy metal type, dose, and plant species, along with their interactions, significantly influenced the relative germination ratio, relative germination index, relative vigor index, relative shoot and root length, as well as fresh and dry biomass accumulation. Among metals, Cd and Hg exhibited the strongest toxicity, markedly reducing germination rates, seedling vigor, and biomass, while Cr, Pb, and Ni caused less inhibition. Species responses varied, with sainfoin and common vetch showing higher tolerance and maintaining better growth performance under metal stress compared to the more sensitive alfalfa. Dose-dependent declines were evident across all traits, highlighting the severity of heavy metal stress at elevated concentrations. These findings emphasize the critical role of species selection for phytoremediation and sustainable cultivation in contaminated soils. Further investigation into the physiological and molecular mechanisms underlying metal tolerance could support improved management strategies in metal-impacted environments.

Keywords: heavy metal toxicity, seed germination, seedling growth, phytoremediation, plant tolerance

Esra Nermin Ertekin, Agric. Eng. Department of Field Crops, Institute of Science, Hatay Mustafa Kemal University, Hatay, Türkiye, email: nermin.kucukors@hotmail.com
* Funded by Akdeniz University Scientific Research Projects Coordination Unit. Project number: FYL-2017-2009.

INTRODUCTION

In recent decades, the intensification of industrial activities has led to a concerning accumulation of pollutants in the environment, particularly in agricultural soils. Among these pollutants, heavy metals have emerged as one of the most persistent and hazardous contaminants due to their non-biodegradable nature and potential to bioaccumulate in ecological systems. Heavy metals such as cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), and lead (Pb) are commonly released into the environment through various anthropogenic sources, including mining operations, industrial discharges, pesticide and fertilizer application, and wastewater irrigation. Once introduced into the soil, these metals can remain for decades, posing a significant threat to plant health, crop productivity, and ultimately, food safety (Can et al. 2009, Nagajyoti et al. 2010, Alloway 2012).

Plants exposed to elevated levels of heavy metals often exhibit a range of physiological and biochemical disturbances. These include inhibited seed germination, stunted seedling growth, reduced root elongation, and disruptions in nutrient uptake and photosynthesis (Singh et al. 2010, Pourrut et al. 2011). On the cellular level, heavy metals can induce oxidative stress by generating excessive reactive oxygen species (ROS), which damage lipids, proteins, and nucleic acids, ultimately impairing membrane integrity and enzymatic activities (Sharma, Dietz, 2006, Gupta et al. 2013). Furthermore, metals such as Cd and Pb interfere with essential nutrient absorption, alter hormonal signaling pathways, and suppress antioxidant defense mechanisms, leading to imbalances in metabolic homeostasis (Seregin, Kozhevnikova 2008). The severity of these effects depends not only on the concentration and type of metal, but also on the plant species and developmental stage. Seed germination and early seedling growth are considered highly sensitive indicators of metal toxicity, and are frequently used as early biomarkers in environmental risk assessments (Zhao et al. 2002).

Leguminous forage crops such as alfalfa (*Medicago sativa* L.), common vetch (*Vicia sativa* L.), and sainfoin (*Onobrychis viciifolia* Scop.) play a vital role in sustainable agriculture due to their high nutritional value, soil fertility improvement through nitrogen fixation, and adaptability to diverse agro-climatic conditions (Atis et al. 2011, Ertekin et al. 2017). However, their early growth stages are particularly vulnerable to environmental stressors, including heavy metal contamination. Despite the ecological and agronomic importance of these species, comparative studies evaluating their responses to different types and concentrations of heavy metals remain limited.

Previous studies have mostly focused on single species or individual metal treatments, leaving a gap in the understanding of interspecific variability in tolerance and physiological responses (Liu et al. 2010, Timm et al. 2019). In this context, exploring the germination and seedling vigor responses of multiple forage legumes under controlled heavy metal stress is essential not only for identifying tolerant species, but also for assessing their potential use in phytoremediation or rehabilitation of contaminated lands.

The present study aims to address this gap by investigating the effects of some heavy metals applied at increasing concentrations on the seed germination and early seedling development of alfalfa, common vetch, and sainfoin. By examining key physiological parameters such as germination percentage, root growth inhibition, and relative germination and growth indices, this research seeks to provide a comparative assessment of heavy metal tolerance among these species. Unlike previous studies that mainly focused on crop toxicity thresholds, this study integrates physiological and comparative perspectives to better elucidate tolerance patterns among forage legumes. The findings are expected to offer insights into the potential of these legumes for cultivation or phytoremediation in contaminated soils, and contribute to risk evaluation strategies in agroecosystems affected by industrial pollution. Ultimately, understanding these mechanisms can support the development of sustainable forage production systems and informed management practices for agriculture in marginal or contaminated environments.

MATERIALS AND METHODS

Experimental design and plant materials

This experiment was conducted under controlled laboratory conditions at the Department of Field Crops, Faculty of Agriculture, Akdeniz University. The study involved three leguminous forage species, alfalfa (*Medicago sativa* L.), common vetch (*Vicia sativa* L.), and sainfoin (*Onobrychis viciifolia* Scop.), as the plant material. Certified seeds of each species were obtained from reliable agricultural research institutions and stored under optimal conditions until use.

Five different heavy metals were tested as stress-inducing agents: cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), and lead (Pb). Their respective compound forms and molecular weights are listed in Table 1. All chemicals were of analytical grade and sourced from commercial suppliers such as Merck, Riedel-de Haën, and PanReac.

Germination assay and treatment application

To ensure aseptic conditions, seeds were initially sterilized by immersion in 5% hydrogen peroxide (H_2O_2) for 3 minutes, followed by five successive rinses with sterile distilled water. This concentration was selected because 5% H_2O_2 effectively eliminates surface-borne pathogens and fungal spores while avoiding detrimental effects on seed viability, as demonstrated in pre-

Table 1

Characteristics of the heavy metals used in the experiment

Heavy Metal	Chemical Form	Molecular Weight (g mol^{-1})	Supplier
Cadmium (Cd)	$Cd(NO_3)_2 \cdot 4H_2O$	308.49	PanReac
Chromium (Cr)	$Cr(NO_3)_3 \cdot 9H_2O$	400.15	Merck
Mercury (Hg)	$Hg(NO_3)_2 \cdot H_2O$	342.62	Merck
Nickel (Ni)	$Ni(NO_3)_2 \cdot 6H_2O$	290.81	Merck
Lead (Pb)	$Pb(NO_3)_2$	331.20	Riedel-de Haën

vious seed disinfection studies (Ertekin et al. 2018, 2022). Uniform seeds were selected for each species, and 30 seeds per replicate were sown in sterile 9 cm Petri dishes lined with double-layered Whatman No. 1 filter papers.

Each Petri dish received 8 mL of heavy metal solution at one of the following concentrations: 0 (control), 100, 200, 400, or 800 mg L^{-1}. These solutions were prepared from 1 L stock solutions of the respective compounds. Treatments were arranged in a completely randomized design with three replicates per treatment for each plant species. Dishes were sealed with parafilm to minimize evaporation and placed in an incubator set at 30 ± 1°C, with a 12-hour light (200±20 μmol m^{-2} s^{-1}) / 12-hour dark cycle and 70% relative humidity. The germination and early growth period lasted 10 days.

Measurement of germination and seedling parameters

A seed was considered germinated when its radicle extended beyond 2 mm. Germination was monitored daily, and the number of germinated seeds was recorded until no further germination was observed. At the end of the experiment, 13 seedlings per replicate were randomly selected to measure root length (RL) and shoot length (SL) using a millimeter-scale ruler.

In addition to germination percentage (GP), several derived indices were calculated to evaluate the effects of heavy metal stress:

– germination rate (GR) was calculated as the proportion of seeds germinated over total seeds per Petri dish.

GR (%)=(number of germinated seeds / total number of seeds)×100;

– mean germination time (MGT) was computed following the method of Ellis and Roberts (1981):

MGT = $\Sigma(D \times n) / \Sigma n$, where D is the number of days and n is the number of seeds germinated on day D.

– index (GI) was determined based on cumulative germination per day.

$$GI=\sum Dt/Gt,$$

where: G_t = number of seeds germinated on day t;

D_t = day number of germination;

– vigor index (VI) was calculated as the product of GI and mean shoot length:

$$VI=GI \times \text{shoot length (cm)};$$

– relative germination index (RGI %), relative vigor index (RVI%), relative root length (RRL %), and relative shoot length (RSL %) were calculated as the ratio of each parameter under heavy metal stress to that of the control, expressed in percentage:

RGI (%)=(GI treatment / GI control)×100,

RVI (%)=(VI treatment / VI control)×100,

RRL (%)=(Root Length treatment / root lengthcontrol)×100,

RSL (%)=(shoot length treatment / shoot length control)×100.

Additionally, root fresh weight (RFW) and shoot fresh weight (SFW) were also recorded at the end of the experiment.

Statistical analysis

The experiment was conducted under controlled laboratory conditions using a completely randomized factorial design, where three legume species, five heavy metals, and five concentration levels (including control) were considered independent factors. All data collected from the experiment were expressed as a means of three replicates.

Statistical analyses were performed using JMP Pro 13.0 (SAS Institute Inc.). The experimental design was analyzed as a full factorial experiment with three fixed factors: species (S), heavy metal type (HM), and dose (D). A three-way analysis of variance (ANOVA) including all main effects (S, HM, D) and their two- and three-way interactions (S×HM, S×D, HM×D, S×HM×D) was applied to the entire dataset in order to test overall treatment effects. When significant differences were detected ($p<0.05$), the Tukey's Honest Significant Difference (HSD) test was applied to compare treatment means. Prior to hypothesis testing, model assumptions were evaluated for each response variable. Normality of residuals was assessed by visual inspection of Q-Q plots and by the Shapiro-Wilk test.

Additionally, to visualize three-way interactions among plant species, heavy metal types, and doses, heatmap graphics were generated in JMP Pro 13.0. These visualizations provided a clear and comparative representation of how germination-related indices varied across all treatment combinations.

RESULTS AND DISCUSSION

Relative germination ratio (%)

According to the ANOVA results (Table 2), heavy metal type and dose had significant effects on the relative germination ratio – RGR ($p<0.0001$),

Table 2

ANOVA results of investigated parameters under main and interaction effects

ITEMS	*P* values of treated factors						
	Main effects			Interaction effects			
	S	HM	D	S×HM	S×D	HM×D	S×HM×D
RGR	0.0936	<0.0001	<0.0001	<0.0001	0.1227	0.0052	0.1504
RGI	0.0002	<0.0001	<0.0001	<0.0001	0.2500	<0.0001	<0.0001
RVI	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
RSL	0.0936	<0.0001	<0.0001	<0.0001	0.1227	0.0052	0.1504
RRL	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MGT	<0.0001	<0.0001	<0.0001	<0.0001	0.4052	0.0030	<0.0001
FSW	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
FRW	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DSW	<0.0001	<0.0001	0.0029	<0.0001	<0.0001	<0.0001	<0.0001
DRW	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

S – species, HM – heavy metals, D – doses, RGR – relative germination ratio (%), RGI – relative germination index (%), RVI – relative vigor index (%), RSL – relative shoot length (%): RRL – relative root length (%), MGT – mean germination time (day), FSW – fresh shoot weight (mg plantlet^{-1}), FRW – Fresh root weight (mg plantlet^{-1}), DSW: Dry shoot weight (mg plantlet^{-1}), DRW – dry root weight (mg plantlet^{-1})

along with significant interactions for HM × D (p=0.0052) and S × HM (p<0.0001). However, species alone and other interactions were not significant. Among metals (Table 3), Cr (100.1 ± 0.6), Pb (100.0 ± 0.8), and Ni (99.4 ± 0.6) resulted in the highest RGRs, whereas Cd (96.1 ± 0.7) and Hg (96.7 ± 0.8) caused greater inhibition. RGR was highest in the control (99.9 ± 0.5), followed closely by 100 and 200 mg L^{-1} treatments, while 800 mg L^{-1} (95.9 ± 1.0) caused the biggest decline. Regarding species – metal combinations (Table 4), sainfoin × Cr (102.9 ± 1.0) and alfalfa × Pb (102.9 ± 1.5) yielded the highest RGRs. The lowest value was found in alfalfa × Cd (92.8 ± 1.3), followed by sainfoin × Hg (93.5 ± 1.6). According to Table 5, Cr × 200 mg L^{-1} (101.9 ± 1.1) had the highest RGR, comparable to several other Cr and Pb combinations, especially at moderate doses. The lowest RGR occurred in Cd × 800 mg L^{-1} (91.2 ± 2.0), with similarly low results in Hg × 800 mg L^{-1} and Cd × 200 mg L^{-1}. These findings confirm that heavy metal type and concentration significantly affect seed germination, which is consistent with previous studies showing that metals like Cd and Hg are more toxic than Cr, Pb, or Ni (Clemens 2006, Ertekin, Bilgen 2011, Ertekin et al. 2020, Aygün et al. 2022). The higher tolerance observed with Cr, Pb, and Ni may be due to species-specific detoxification mechanisms (Sharma, Dubey 2005). The reduction in germination at higher doses (400 and 800 mg L^{-1}) aligns with the known dose-dependent toxicity effects impairing seed metabolic activity (Pourrut et al. 2011). Species-metal interactions high-

Table 3

Effects of species and heavy metals, and doses on the parameters investigated

Factors	Levels	ITEMS									
		RGR	RGI	RVI	RSL	RRL	MGT	FSW	FRW	DSW	DRW
Species	Alfalfa	98.2±0.7	97.0±1.1 ^{a}	66.6±4.1 ^{b}	67.0±4.0	63.6±3.1 ^{b}	1.3±0.0 ^{c}	25.9±0.9 ^{c}	9.1±0.3 ^{c}	1.9±0.1 ^{c}	1.2±0.1 ^{c}
	Vetch	99.3±0.2	93.6±0.9 ^{b}	67.8±2.9 ^{b}	71.0±2.9	58.4±3.6 ^{c}	1.9±0.0 ^{b}	40.5±1.4 ^{b}	38.2±1.7 ^{a}	4.1±0.1 ^{b}	4.0±0.1 ^{a}
	Sainfoin	97.9±0.7	96.8±0.8 ^{a}	76.8±2.8 ^{a}	78.3±2.6	70.2±3.1 ^{a}	2.2±0.0 ^{a}	75.9±1.1 ^{a}	21.1±0.6 ^{b}	12.1±0.2 ^{a}	2.3±0.1 ^{b}
Heavy Metals	Cd	96.1±0.7 ^{b}	91.8±1.4 ^{d}	54.2±4.4 ^{c}	56.7±4.4 ^{d}	42.0±4.3 ^{e}	1.9±0.1 ^{a}	42.0±3.2 ^{d}	21.8±2.0 ^{b}	6.3±0.7 ^{b}	2.7±0.2 ^{ab}
	Cr	100.1±0.6 ^{a}	95.9±1.1 ^{bc}	93.4±2.6 ^{a}	96.8±2.2 ^{a}	96.7±2.4 ^{a}	1.7±0.0 ^{c}	55.5±2.6 ^{a}	24.8±1.8 ^{a}	5.8±0.5 ^{c}	2.1±0.2 ^{c}
	Hg	96.7±0.8 ^{b}	93.2±1.4 ^{cd}	42.6±4.5 ^{d}	44.2±4.4 ^{e}	52.6±4.1 ^{d}	1.8±0.1 ^{bc}	39.0±3.3 ^{e}	16.7±1.3 ^{c}	5.4±0.7 ^{d}	2.2±0.1 ^{c}
	Ni	99.4±0.6 ^{a}	98.2±0.9 ^{ab}	73.8±3.4 ^{b}	75.0±3.2 ^{c}	59.8±3.5 ^{c}	1.8±0.1 ^{b}	48.3±2.5 ^{c}	25.2±2.6 ^{a}	6.7±0.6 ^{a}	2.6±0.3 ^{b}
	Pb	100.0±0.8 ^{a}	100.1±1.0 ^{a}	88.0±2.0 ^{a}	88.0±1.8 ^{b}	69.4±3.1 ^{b}	1.9±0.1 ^{bc}	52.3±3.4 ^{b}	25.5±1.9 ^{a}	5.9±0.5 ^{c}	2.8±0.2 ^{a}
Doses (mg L^{-1})	100	99.2±0.6 ^{a}	97.7±0.9 ^{ab}	75.8±3.4 ^{b}	77.1±3.2 ^{b}	69.6±2.8 ^{b}	1.8±0.1 ^{bc}	48.5±2.8 ^{b}	23.8±2.0 ^{b}	5.9±0.6 ^{ab}	2.6±0.2 ^{b}
	200	99.3±0.7 ^{a}	95.8±1.1 ^{b}	66.8±4.1 ^{c}	68.9±4.0 ^{c}	61.9±3.6 ^{c}	1.8±0.1 ^{b}	45.7±3.0 ^{c}	21.8±1.7 ^{c}	5.8±0.6 ^{b}	2.4±0.2 ^{bc}
	400	98.0±0.8 ^{ab}	95.7±1.1 ^{b}	60.0±4.4 ^{d}	61.9±4.4 ^{d}	50.8±4.0 ^{d}	1.8±0.1 ^{bc}	45.3±3.4 ^{c}	20.5±1.6 ^{cd}	5.9±0.6 ^{ab}	2.2±0.2 ^{cd}
	800	95.9±1.0 ^{b}	89.9±1.6 ^{c}	49.3±4.4 ^{e}	52.7±4.5 ^{e}	38.2±4.0 ^{e}	1.9±0.1 ^{a}	43.7±3.6 ^{c}	18.6±1.5 ^{d}	6.1±0.7 ^{a}	2.2±0.2 ^{d}
	Control	99.9±0.5 ^{a}	100.0±0.8 ^{a}	100.0±1.3 ^{a}	100.0±1.1 ^{a}	100.0±1.5 ^{a}	1.7±0.1 ^{c}	53.9±2.6 ^{a}	29.4±2.8 ^{a}	6.2±0.5 ^{a}	2.9±0.2 ^{a}

Cd – cadmium, Cr – chromium, Hg – mercury, Ni – nickel, Pb – lead, RGR – relative germination ratio (%), RGI – relative germination index (%), RVI – relative vigor index (%), RSL – relative shoot length (%), RRL – relative root length (%), MGT – mean germination time (day), FSW – Fresh shoot weight (mg $plantlet^{-1}$), FRW – fresh root weight (mg $plantlet^{-1}$), DSW – dry shoot weight (mg $plantlet^{-1}$), DRW – dry root weight (mg $plantlet^{-1}$)

Values shown with different superscripts in the same column are significantly different from each other.

Table 4

Effects of species and heavy metal interactions on the parameters investigated

Species	Heavy Metals	ITEMS									
		RGR	RGI	RVI	RSL	RRL	MGT	FSW	FRW	DSW	DRW
Alfalfa	Cd	$92.8±1.3^{d}$	$86.1±3.4^{h}$	$34.6±8.6^{i}$	$36.0±8.6^{h}$	$41.8±7.2^{ef}$	$1.4±0.1^{e}$	$16.9±2.4^{i}$	$10.0±1.3^{g}$	$1.6±0.2^{gh}$	$1.6±0.3^{g}$
	Cr	$96.7±1.2^{bcd}$	$97.0±1.5^{c\text{-}f}$	$92.0±3.3^{bc}$	$94.9±3.0^{abc}$	$91.8±4.5^{ab}$	$1.3±0.0^{ef}$	$32.1±0.5^{gh}$	$10.1±0.3^{g}$	$2.0±0.0^{fg}$	$0.8±0.0^{h}$
	Hg	$98.5±1.5^{abc}$	$99.3±1.3^{a\text{-}d}$	$33.5±8.0^{i}$	$33.5±8.0^{h}$	$62.2±6.6^{c}$	$1.1±0.0^{g}$	$18.8±1.6^{i}$	$9.4±0.8^{g}$	$1.1±0.1^{h}$	$2.0±0.1^{g}$
	Ni	$99.9±1.2^{ab}$	$100.5±1.7^{abc}$	$72.3±8.5^{efg}$	$72.1±8.1^{ef}$	$58.1±6.5^{cd}$	$1.2±0.0^{fg}$	$29.6±0.6^{h}$	$7.8±0.5^{g}$	$2.3±0.1^{f}$	$0.7±0.1^{h}$
	Pb	$102.9±1.5^{a}$	$102.1±2.2^{a}$	$100.5±2.2^{ab}$	$98.6±1.2^{ab}$	$64.2±4.8^{c}$	$1.2±0.0^{g}$	$31.9±0.6^{gh}$	$8.1±0.3^{g}$	$2.2±0.1^{fg}$	$0.8±0.1^{h}$
Vetch	Cd	$99.4±0.3^{ab}$	$93.9±1.0^{ef}$	$69.7±4.8^{fgh}$	$73.7±4.5^{ef}$	$35.2±7.6^{f}$	$2.0±0.0^{c}$	$37.4±2.0^{fg}$	$35.8±4.3^{c}$	$4.1±0.2^{e}$	$3.9±0.3^{c}$
	Cr	$100.6±0.3^{ab}$	$89.5±2.0g^{h}$	$83.1±4.5^{cde}$	$91.5±4.1^{bc}$	$98.3±4.5^{a}$	$1.8±0.0^{d}$	$56.6±1.9^{d}$	$41.9±1.9^{b}$	$4.8±0.2^{d}$	$3.7±0.2^{cd}$
	Hg	$98.0±0.7^{abc}$	$87.0±2.7^{h}$	$33.2±8.0^{i}$	$35.1±7.9^{h}$	$38.5±7.7^{ef}$	$2.0±0.1^{c}$	$26.6±3.7^{h}$	$22.7±3.2^{e}$	$2.2±0.3^{fg}$	$2.6±0.3^{ef}$
	Ni	$100.0±0.4^{ab}$	$97.3±1.0^{b\text{-}e}$	$74.7±3.6^{def}$	$76.6±3.4^{de}$	$63.1±5.3^{c}$	$2.0±0.0^{c}$	$43.5±2.4^{e}$	$50.1±3.4^{a}$	$4.9±0.2^{d}$	$5.2±0.3^{a}$
	Pb	$98.4±0.6^{abc}$	$100.1±1.1^{abc}$	$78.2±3.0^{def}$	$78.2±3.0^{de}$	$57.0±5.6^{cd}$	$2.0±0.0^{c}$	$38.3±1.3^{ef}$	$40.4±2.4^{b}$	$4.4±0.1^{de}$	$4.4±0.1^{b}$
Sainfoin	Cd	$96.1±1.1^{bcd}$	$95.3±1.6^{def}$	$58.2±7.1^{h}$	$60.2±7.1^{g}$	$49.1±7.4^{de}$	$2.2±0.0^{ab}$	$71.8±2.2^{c}$	$19.5±1.0e^{f}$	$13.0±0.3^{a}$	$2.7±0.1^{e}$
	Cr	$102.9±1.0^{a}$	$101.2±1.1^{ab}$	$104.9±4.1^{a}$	$103.9±3.6^{a}$	$100.0±3.6^{a}$	$2.2±0.0^{b}$	$77.8±1.3^{b}$	$22.4±0.7^{e}$	$10.5±0.2^{c}$	$1.8±0.1^{g}$
	Hg	$93.5±1.6^{cd}$	$93.2±2.1^{fg}$	$61.0±5.6^{gh}$	$64.1±5.0^{fg}$	$56.9±6.2^{cd}$	$2.3±0.0^{a}$	$71.5±0.9^{c}$	$18.0±0.8^{f}$	$12.7±0.2^{a}$	$2.1±0.1^{fg}$
	Ni	$98.4±1.1^{abc}$	$96.7±1.6^{c\text{-}f}$	$74.5±5.0^{def}$	$76.3±4.4^{e}$	$58.4±6.5^{cd}$	$2.2±0.0^{ab}$	$71.8±1.5^{c}$	$17.6±0.9^{f}$	$12.9±0.2^{a}$	$1.7±0.1^{g}$
	Pb	$98.7±1.7^{ab}$	$97.9±1.7^{b\text{-}e}$	$85.5±3.0^{cd}$	$87.2±2.8^{cd}$	$86.8±2.9^{b}$	$2.2±0.0^{b}$	$86.6±3.4^{a}$	$27.9±1.2^{d}$	$11.1±0.5^{b}$	$3.2±0.3^{d}$

Cd – cadmium, Cr – chromium, Hg – mercury, Ni – Nickel, Pb – lead, RGR – relative germination ratio (%), RGI – relative germination index (%), RVI – relative vigor index (%), RSL – relative shoot length (%), RRL – relative root length (%), MGT – Mean germination time (day), FSW – fresh shoot weight (mg plantlet^{-1}), FRW – fresh root weight (mg plantlet^{-1}), DSW – dry shoot weight (mg plantlet^{-1}), DRW – dry root weight (mg plantlet^{-1})

Values shown with different superscripts in the same column are significantly different from each other.

Table 5

Effects of species and dose interactions on the parameters investigated

Species	Doses (mg L^{-1})	ITEMS									
		RGR	RGI	RVI	RSL	RRL	MGT	FSW	FRW	DSW	DRW
Alfalfa	100	98.1±1.4	99.9±2.0	77.7±7.9^{bcd}	76.9±7.2	71.8±4.6^{bc}	1.2±0.0	28.0±1.1^{fg}	8.4±0.6^{h}	2.0±0.1^{e}	1.0±0.1^{i}
	200	99.0±1.4	97.8±1.8	65.3±9.3^{ef}	66.4±9.3	64.5±5.7^{cd}	1.3±0.0	25.7±1.8^{gh}	9.3±0.4^{h}	1.8±0.1^{e}	1.1±0.1^{hi}
	400	97.4±1.5	96.4±1.5	50.4±8.5^{gh}	51.8±8.6	50.6±6.3^{ef}	1.2±0.0	23.1±2.1^{gh}	9.5±0.6^{h}	1.9±0.1^{e}	1.2±0.1^{hi}
	800	96.5±2.0	91.1±4.0	39.5±9.4^{h}	39.9±9.5	31.4±3.7^{h}	1.3±0.1	21.1±2.9^{h}	9.7±1.3^{h}	1.6±0.2^{e}	1.6±0.3^{gh}
	Control	99.9±1.4	100.0±1.9	100.0±2.6^{a}	100.0±1.9	100.0±2.4^{a}	1.2±0.0	31.4±0.6^{ef}	8.7±0.6^{h}	1.9±0.1^{e}	1.1±0.1^{hi}
Vetch	100	99.6±0.4	94.0±1.4	66.7±4.8^{def}	70.8±5.0	57.3±5.1^{de}	1.9±0.0	42.0±2.2^{c}	39.9±3.2^{b}	4.2±0.2^{e}	4.2±0.3^{b}
	200	99.6±0.4	91.6±2.4	55.6±4.9^{fg}	59.8±5.0	51.9±7.2^{ef}	2.0±0.0	35.6±2.7^{de}	35.5±2.9^{bc}	3.8±0.3^{e}	3.9±0.3^{bc}
	400	98.8±0.7	93.1±2.0	61.3±6.9^{efg}	64.1±7.1	44.2±7.2^{fg}	1.9±0.0	37.3±3.7^{cd}	31.8±2.7^{cd}	3.8±0.3^{e}	3.5±0.3^{c}
	800	98.4±0.7	89.2±2.0	55.3±6.5^{fg}	60.4±7.1	38.7±8.4^{gh}	2.0±0.0	35.3±4.1^{de}	27.4±3.0^{de}	3.7±0.3^{e}	3.0±0.3^{d}
	Control	100.0±0.3	100.0±1.4	100.0±1.8^{a}	100.0±1.9	100.0±2.5^{a}	1.8±0.0	52.2±1.1^{b}	56.4±3.1^{b}	5.0±0.1^{c}	5.2±0.2^{a}
Sainfoin	100	99.8±1.1	99.2±1.1	83.0±4.1^{b}	83.5±3.8	79.7±3.4^{b}	2.2±0.0	75.4±1.5^{a}	23.1±1.4^{a}	11.6±0.4^{b}	2.7±0.3^{de}
	200	99.4±1.4	98.1±1.4	79.5±5.5^{bc}	80.5±4.9	69.5±5.4^{bc}	2.2±0.0	75.9±1.3^{a}	20.5±0.9^{a}	11.9±0.3^{b}	2.3±0.2^{ef}
	400	97.9±1.7	97.5±1.9	68.5±7.3^{cde}	69.7±7.0	57.5±7.5^{de}	2.2±0.0	75.5±3.1^{a}	20.1±1.3^{a}	12.1±0.3^{b}	2.1±0.2^{fg}
	800	92.8±1.8	89.3±2.1	53.1±6.3^{g}	57.9±6.1	44.5±7.6^{fg}	2.3±0.0	74.7±3.9^{a}	18.7±1.7^{a}	13.1±0.4^{a}	2.0±0.2^{fg}
	Control	99.8±0.6	100.0±0.8	100.0±2.4^{a}	100.0±2.2	100.0±3.0^{a}	2.2±0.0	78.1±1.3^{a}	23.0±0.5^{a}	11.6±0.3^{b}	2.5±0.1^{def}

RGR – relative germination ratio (%), RGI – relative germination index (%), RVI – relative vigor index (%), RSL – relative shoot length (%): RRL – relative root length (%), MGT – mean germination time (day), FSW – fresh shoot weight (mg plantlet^{-1}), FRW – fresh root weight (mg plantlet^{-1}), DSW – dry shoot weight (mg plantlet^{-1}), DRW – dry root weight (mg plantlet^{-1})
Values shown with different superscripts in the same column are significantly different from each other.

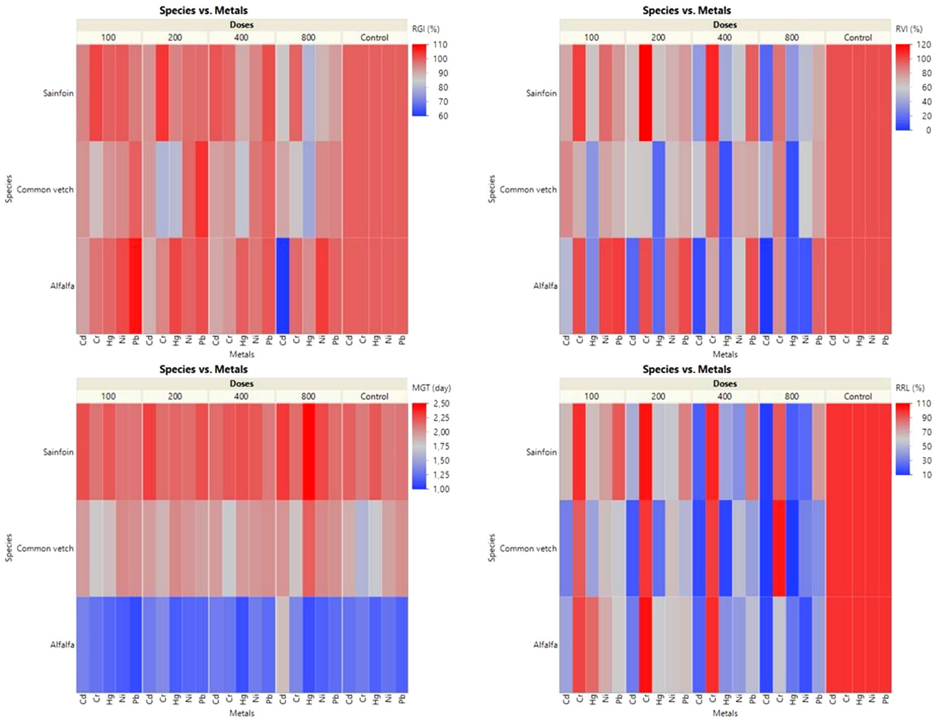

Fig. 1. Heatmaps of three-way interactions (species × metals × doses) for RRL, RGI, RVI and MGT parameters

light variability in susceptibility, which is crucial for risk assessment and phytoremediation planning (Ghosh, Singh 2005). Overall, these results emphasize the need to consider both metal type and dose when evaluating heavy metal impacts on plant germination.

Relative germination index (%)

According to the ANOVA results (Table 2), species (S), heavy metal type (HM), and dose (D) had significant effects on the relative germination index – RGI (p=0.0002, p<0.0001, and p<0.0001, respectively). Significant interactions were also observed for S × HM, HM × D, and S × HM × D (p<0.0001 for all), while S × D was not significant. Among species (Table 3), alfalfa (97.0 ± 1.1) and sainfoin (96.8 ± 0.8) had higher RGI values, while vetch (93.6 ± 0.9) scored lower RGI. Pb (100.1 ± 1.0), Ni (98.2 ± 0.9), and Cr (95.9 ± 1.1) caused the least inhibition, whereas Cd (91.8 ± 1.4) and Hg (93.2 ± 1.4) showed more toxic effects. Control (100.0 ± 0.8), 100 mg L^{-1} (97.7 ± 0.9), and 200 mg L^{-1} (95.8 ± 1.1) had higher RGI than 800 mg L^{-1} (89.9 ± 1.6), indicating a dose-dependent decline. As shown in Figure 1, the heatmap illustrates species-specific responses under different metal types and doses, ranging from high (~110%) to low RGI values (~60%). Alfalfa and sainfoin generally maintained higher RGI under moderate stress, while vetch showed greater sensitivity. These results demonstrate that species, metal type, and dose strongly affect RGI. Alfalfa and sainfoin showed better tolerance than vetch, consistent with their higher resilience to environmental stress (Sharma and Dubey, 2005). Pb, Ni, and Cr were less inhibitory than Cd and Hg, supporting prior findings on metal toxicity (Clemens, 2006). The dose-dependent decline in RGI aligns with the known thresholds for toxicity affecting seedling vigor (Ertekin, Bilgen, 2011, Ertekin et al. 2020, Aygün et al. 2022). Species-specific differences suggest that selecting the right plant species is crucial for phytoremediation and cultivation in contaminated environments.

Relative vigor index (%)

As shown in Table 2, the species, heavy metal type, and dose, along with all their interactions, significantly affected the relative vigor index – RVI (p<0.0001). According to Table 3, sainfoin had the highest RVI (76.8 ± 2.8%), while alfalfa showed the lowest value (66.6 ± 4.1%). Among metals, Cr yielded the highest RVI (93.4 ± 2.6%), while Hg resulted in the lowest value (42.6 ± 4.5%). RVI values declined progressively with increasing metal doses. The heatmap (Figure 1) of species × heavy metal × dose interactions revealed that control treatments showed consistently higher vigor. Notably, Hg application to alfalfa led to strong reductions across all doses, indicating the most toxic response. The significant effects of species, heavy metal type, and dose on relative vigor index (RVI) reflect the combined influence of genetic and environmental stress factors on seedling vigor, consistent with prior studies (Ertekin, Bilgen 2011, Bae et al. 2016, Ertekin et al. 2020, Aygün et al. 2022). Sainfoin demonstrated the highest vigor, suggesting inherent tolerance mechanisms, whereas alfalfa showed comparatively lower vigor, indicating species-specific sensitivity. Chromium (Cr) treatment resulted in the highest RVI, supporting earlier findings that Cr, at certain valence states and concentrations, may be less phytotoxic than mercury (Hg), which consistently caused the lowest RVI values across species and doses (Di Toppi, Gabbrielli, 1999, Clemens, 2006). The observed dose-dependent decline in vigor confirms the detrimental impact of increasing heavy metal concentrations on seedling metabolic functions and growth potential (Pourrut et al. 2011). Particularly, the pronounced negative effect of Hg on alfalfa emphasizes the need for careful consideration of species-specific responses in heavy metal-contaminated environments and phytoremediation efforts (Ghosh, Singh 2005).

Relative shoot length (%)

Relative shoot length (RSL) was significantly affected by heavy metal type and dose (p<0.0001), while species alone had no significant effect (p=0.0936), although the interaction S × HM was significant (p<0.0001), indicating variable species responses to metals. The HM × D interaction was also significant (p=0.0052), while others were not. As shown in Table 3, sainfoin showed the highest numerical RSL (78.3 ± 2.6%), although differences among species were not statistically significant. Cr (96.8 ± 2.2%) and Pb (88.0 ± 1.8%) resulted in the highest RSL, while Hg (44.2 ± 4.4%) and Cd (56.7 ± 4.4%) caused the greatest reductions. Control (100.0 ± 1.1%) maintained the highest shoot length; 800 mg L^{-1} resulted in the lowest (52.7 ± 4.5%). Table 4 revealed species-specific responses. Sainfoin showed maximum RSL under Cr (103.9 ± 3.6%), while alfalfa had lowest values under Hg (33.5 ± 8.0%) and Cd (36.0 ± 8.6%). Vetch ranged from 35.1% under Hg to 91.5% under Cr. According to Table 6, all metals showed dose-dependent declines. Cd decreased sharply from 73.8% to 23.3%, Hg from 43.1% to 21.2%, while Cr maintained ~95–100% even at higher doses. Ni and Pb also showed moderate tolerance. RSL was significantly influenced by heavy metal type and dose, while species effect alone was not significant, suggesting that metal toxicity primarily governs shoot growth inhibition during early seedling development (Clemens 2006, Ertekin, Bilgen 2011, Ertekin et al. 2020, Aygün et al. 2022). The higher RSL values under chromium (Cr) and lead (Pb) treatments indicate comparatively lower toxicity, consistent with reports that these metals, at certain concentrations, exert less phytotoxicity than cadmium (Cd) and mercury (Hg) (Di Toppi, Gabbrielli 1999, Sharma, Dubey 2005). The strong reduction in shoot length observed under Cd and Hg, particularly at higher doses, aligns with their known interference with cell division and elongation processes (Pourrut et al. 2011). Species-specific responses, such as sainfoin maintaining higher RSL values, may be related to intrinsic tolerance mechanisms or more effective detoxification pathways (Bae et al. 2015). The dose-dependent decline in RSL across all metals underscores the progressive severity of heavy metal stress on seedling growth, emphasizing the need for dose consideration in environmental risk assessments and phytoremediation planning.

Relative root length (%)

ANOVA results (Table 2) showed that species, heavy metal type, and dose significantly affected relative root length – RRL (p<0.0001 for all), along with all interaction terms. Sainfoin had the highest RRL (70.2%), followed by alfalfa (63.6%) and vetch (58.4%) – Table 3. Cr caused the least reduction in root length (96.7%), while Cd was most toxic (42.0%). RRL decreased progressively with higher doses, dropping to 38.2% at 800 mg L^{-1}. The heatmap (Figure 1) revealed that sainfoin maintained high RRL under most treatments, particularly under Cr and Pb, while alfalfa showed the lowest RRL values under Cd and Hg, indicating species- and metal-specific root responses. The significant effects of species, heavy metal type, and dose on RRL, along with all interaction effects, underscore the complexity of root responses to metal stress. Sainfoin exhibited the highest root growth under heavy metal exposure, indicating strong tolerance mechanisms, possibly via efficient

Table 6

Effects of heavy metal and dose interactions on the parameters investigated

Heavy Metals	Doses (mg L^{-1})	ITEMS									
		RGR	RGI	RVI	RSL	RRL	MGT	FSW	FRW	DSW	DRW
Cd	100	$97.3\pm1.1^{a-d}$	93.7 ± 1.0^{abc}	69.3 ± 5.8^{de}	73.8 ± 5.9^{de}	46.8 ± 4.7^{fgh}	1.85 ± 0.1^{bc}	$48.9\pm6.9^{b-e}$	$20.7\pm3.7^{e-j}$	$6.4\pm1.3^{a-e}$	$2.5\pm0.4^{c-g}$
	200	$95.2\pm1.3^{a-d}$	91.8 ± 1.5^{abc}	47.7 ± 7.0^{fg}	51.5 ± 7.3^{fg}	31.8 ± 3.5^{hij}	1.86 ± 0.1^{bc}	44.2 ± 7.8^{ef}	$18.7\pm2.8^{f-j}$	$6.4\pm1.5^{a-d}$	$2.6\pm0.3^{b-g}$
	400	$97.0\pm1.1^{a-d}$	94.6 ± 1.9^{ab}	32.9 ± 6.2^{gh}	34.8 ± 6.7^{hi}	19.4 ± 1.0^{jk}	1.81 ± 0.1^{bcd}	35.2 ± 6.1^{ghi}	16.7 ± 2.3^{hij}	$6.1\pm1.4^{b-f}$	$2.2\pm0.3^{d-i}$
	800	91.2 ± 2.0^{d}	78.6 ± 4.4^{d}	20.9 ± 5.9^{h}	23.3 ± 6.5^{i}	12.1 ± 0.6^{k}	2.05 ± 0.1^{a}	28.8 ± 7.5^{i}	$18.3\pm0.9^{f-j}$	$5.9\pm1.9^{c-h}$	3.0 ± 0.2^{abc}
	Control	99.9 ± 0.6^{ab}	100.0 ± 1.3^{ab}	100.0 ± 2.7^{a}	100.0 ± 2.3^{a}	100.0 ± 2.8^{a}	1.78 ± 0.1^{bcd}	53.1 ± 6.1^{abc}	34.4 ± 8.1^{a}	6.5 ± 1.3^{abc}	3.3 ± 0.7^{a}
Cr	100	99.4 ± 1.8^{ab}	96.0 ± 2.7^{ab}	92.6 ± 6.4^{ab}	95.3 ± 5.1^{ab}	95.7 ± 3.6^{ab}	1.72 ± 0.1^{cd}	52.8 ± 5.5^{abc}	$23.8\pm4.3^{d-g}$	$5.7\pm1.1^{c-i}$	$2.1\pm0.4^{f-i}$
	200	101.9 ± 1.1^{a}	93.2 ± 3.3^{abc}	94.5 ± 8.4^{ab}	99.8 ± 6.7^{a}	103.6 ± 5.9^{a}	1.80 ± 0.1^{bcd}	54.2 ± 5.7^{abc}	$23.7\pm4.1^{d-g}$	$5.6\pm1.1^{d-i}$	$2.0\pm0.4^{f-i}$
	400	98.3 ± 2.0^{abc}	95.2 ± 1.9^{ab}	93.4 ± 5.8^{ab}	97.6 ± 5.0^{ab}	100.0 ± 4.7^{a}	1.74 ± 0.1^{cd}	55.5 ± 6.0^{ab}	26.1 ± 4.2^{cde}	$5.5\pm0.9^{e-i}$	$2.2\pm0.3^{e-i}$
	800	101.1 ± 0.8^{ab}	94.9 ± 2.2^{ab}	86.3 ± 3.1^{ab}	91.1 ± 3.6^{abc}	84.1 ± 7.4^{bc}	1.77 ± 0.1^{bcd}	59.0 ± 6.1^{a}	26.9 ± 4.9^{bcd}	$5.9\pm1.0^{c-h}$	$2.1\pm0.4^{f-i}$
	Control	99.7 ± 0.7^{ab}	100.0 ± 2.0^{ab}	100.0 ± 3.5^{abc}	100.0 ± 3.3^{a}	100.0 ± 3.6^{a}	1.67 ± 0.1^{d}	56.0 ± 6.1^{ab}	$23.5\pm3.5^{d-g}$	$6.0\pm1.2^{b-g}$	$2.1\pm0.3^{f-i}$
Hg	100	99.3 ± 0.7^{ab}	97.6 ± 1.6^{ab}	42.3 ± 5.4^{a}	43.1 ± 5.2^{gh}	65.2 ± 6.1^{de}	1.75 ± 0.1^{bcd}	41.6 ± 6.7^{efg}	$17.7\pm1.8^{g-j}$	$5.5\pm1.6^{f-i}$	$2.3\pm0.2^{c-h}$
	200	$98.0\pm1.7^{a-d}$	93.2 ± 3.2^{abc}	32.5 ± 7.3^{g}	34.4 ± 7.4^{hi}	47.0 ± 4.7^{fgh}	1.79 ± 0.1^{bcd}	37.0 ± 7.8^{fgh}	15.8 ± 1.4^{ijk}	5.1 ± 1.6^{hi}	$2.1\pm0.1^{f-i}$
	400	94.7 ± 1.7^{bcd}	91.1 ± 3.1^{bc}	20.5 ± 4.9^{gh}	22.4 ± 5.3^{i}	31.1 ± 3.9^{ij}	1.78 ± 0.2^{bcd}	32.7 ± 8.4^{hi}	14.6 ± 1.0^{jk}	5.1 ± 1.7^{ghi}	1.9 ± 0.2^{ghi}
	800	91.3 ± 2.4^{cd}	84.0 ± 3.2^{cd}	17.5 ± 3.9^{h}	21.2 ± 4.8^{i}	19.5 ± 2.3^{jk}	1.93 ± 0.2^{ab}	30.3 ± 7.8^{hi}	10.2 ± 0.8^{k}	$5.3\pm1.8^{f-i}$	1.6 ± 0.2^{hi}
	Control	100.0 ± 1.4^{ab}	100.0 ± 1.8^{ab}	100.0 ± 2.7^{h}	100.0 ± 3.2^{a}	100.0 ± 3.0^{a}	1.71 ± 0.1^{cd}	53.3 ± 5.4^{abc}	25.2 ± 5.5^{cde}	$5.8\pm1.4^{c-i}$	3.3 ± 0.4^{ab}
Ni	100	99.9 ± 1.4^{ab}	99.5 ± 2.1^{ab}	90.3 ± 5.6^{a}	90.3 ± 4.1^{abc}	70.2 ± 1.6^{cd}	1.78 ± 0.1^{bcd}	52.4 ± 5.8^{abc}	30.3 ± 7.1^{abc}	7.0 ± 1.4^{a}	3.0 ± 0.7^{abc}
	200	99.8 ± 0.9^{ab}	98.9 ± 0.7^{ab}	75.3 ± 3.7^{abc}	76.2 ± 3.6^{cde}	61.6 ± 1.5^{def}	1.78 ± 0.1^{bcd}	44.6 ± 5.9^{def}	26.6 ± 5.7^{cde}	$6.5\pm1.3^{a-d}$	$2.9\pm0.7^{a-e}$
	400	99.2 ± 1.4^{ab}	96.6 ± 1.9^{ab}	63.0 ± 4.1^{cde}	65.0 ± 3.6^{ef}	43.3 ± 2.5^{ghi}	1.84 ± 0.1^{bc}	47.1 ± 5.5^{cde}	$21.3\pm4.6^{d-i}$	6.8 ± 1.4^{ab}	$2.4\pm0.6^{c-g}$
	800	98.5 ± 1.3^{ab}	95.9 ± 2.3^{ab}	40.4 ± 6.9^{ef}	43.4 ± 7.4^{gh}	24.1 ± 2.5^{jk}	1.83 ± 0.1^{bcd}	42.6 ± 4.8^{efg}	14.9 ± 3.1^{jk}	$6.5\pm1.4^{a-d}$	1.6 ± 0.4^{i}
	Control	99.7 ± 1.3^{ab}	100.0 ± 2.1^{ab}	100.0 ± 3.3^{g}	100.0 ± 2.1^{a}	100.0 ± 3.7^{a}	1.77 ± 0.1^{cd}	54.8 ± 5.7^{ab}	32.9 ± 7.3^{ab}	6.8 ± 1.3^{ab}	3.0 ± 0.7^{abc}
Pb	100	100.0 ± 1.7^{ab}	101.6 ± 2.2^{a}	84.4 ± 4.9^{a}	82.8 ± 3.9^{bcd}	69.9 ± 4.8^{cd}	1.74 ± 0.1^{cd}	46.5 ± 6.3^{cde}	26.5 ± 4.2^{cde}	5.0 ± 0.9^{i}	3.2 ± 0.6^{ab}
	200	101.6 ± 1.4^{ab}	101.9 ± 2.2^{a}	$83.9\pm4.2^{a-d}$	82.7 ± 4.3^{bcd}	65.7 ± 4.0^{de}	1.80 ± 0.1^{bcd}	$48.6\pm6.^{7b-e}$	24.0 ± 3.7^{def}	$5.5\pm1.1^{a-i}$	$2.6\pm0.5^{a-g}$
	400	101.1 ± 2.0^{ab}	100.9 ± 2.1^{ab}	$90.4\pm4.2^{a-d}$	89.7 ± 4.0^{abc}	59.9 ± 5.8^{def}	1.78 ± 0.1^{bcd}	56.0 ± 9.2^{ab}	$23.5\pm3.4^{d-g}$	$6.1\pm1.3^{b-f}$	$2.6\pm0.5^{b-g}$
	800	$97.3\pm2.4^{a-d}$	95.9 ± 2.6^{ab}	81.4 ± 4.2^{abc}	84.7 ± 3.3^{bcd}	51.1 ± 6.1^{efg}	1.80 ± 0.1^{bcd}	57.9 ± 9.8^{a}	$22.6\pm3.4^{d-h}$	7.1 ± 1.6^{a}	$2.7\pm0.5^{a-f}$
	Control	100.1 ± 1.4^{ab}	100.0 ± 2.1^{ab}	100.0 ± 2.8^{bcd}	100.0 ± 1.9^{a}	100.0 ± 4.1^{a}	1.78 ± 0.1^{bcd}	$52.3\pm6.1^{a-d}$	30.8 ± 6.5^{abc}	$5.7\pm1.0^{c-i}$	$2.9\pm0.5^{a-d}$

RGR – relative germination ratio (%), RGI – relative germination index (%), RVI – relative vigor index (%), RSL – relative shoot length (%): RRL – relative root length (%), MGT – mean germination time (day), FSW – fresh shoot weight (mg $plantlet^{-1}$), FRW – fresh root weight (mg $plantlet^{-1}$), DSW – dry shoot weight (mg $plantlet^{-1}$), DRW – dry root weight (mg $plantlet^{-1}$)

Values shown with different superscripts in the same column are significantly different from each other.

metal sequestration or antioxidant defense (Ghosh, Singh 2005, Goncharuk, Zagoskina 2023). In contrast, alfalfa showed greater sensitivity, reflected by the lowest RRL values, consistent with earlier findings that species differ in root susceptibility to metal toxicity (Di Toppi and Gabbrielli, 1999). Cadmium (Cd) was the most toxic metal, significantly reducing root length, while chromium (Cr) had the least adverse effect, supporting previous research on metal-specific toxicity gradients (Clemens 2006, Ertekin and Bilgen, 2011, Ertekin et al. 2020). The dose-dependent decline in RRL across species highlights the importance of considering both concentration and species traits when assessing heavy metal impacts on root development and plant establishment in contaminated soils.

Mean germination time (day)

Mean germination time (MGT) was significantly affected by species, heavy metal type, and dose (p<0.0001 for all), as well as their interactions: S×HM, HM×D, and S×HM×D (all p<0.0001 or p=0.0030). The S×D interaction was not significant (p=0.4052), indicating similar dose effects across species. Alfalfa had the shortest MGT (1.3 ± 0.0 days), followed by vetch (1.9 ± 0.0 days), while sainfoin germinated more slowly (2.2 ± 0.0 days) – Table 3. Among metals, Cr treatment led to the fastest germination (1.7 ± 0.0 days), while Cd and Pb increased MGT to 1.9 ± 0.1 days. The highest dose (800 mg L^{-1}) caused a delay in germination (1.9 ± 0.1 days), compared to the control (1.7 ± 0.1 days). Intermediate doses did not follow a consistent trend. The heatmap (Figure 1) shows MGT values by species, metal, and dose. Alfalfa exhibited fast germination (blue tones) across treatments, while sainfoin showed the longest MGT (dark red), especially under high-dose stress. Vetch presented intermediate responses. These patterns confirm that germination speed is jointly regulated by species characteristics, metal type, and metal concentration. The results indicate that MGT is significantly affected by species, heavy metal type, and metal dose, with notable interactions among these factors. Alfalfa exhibited the shortest MGT, reflecting a faster germination rate and greater tolerance to heavy metal stress, whereas sainfoin showed delayed germination, especially at higher metal concentrations. These species-specific differences align with previous findings that genetic factors strongly influence seed germination speed under abiotic stress (Bae et al. 2015, Goncharuk, Zagoskina 2023). Cr treatment consistently shortened MGT, suggesting lower toxicity or potential stimulatory effects at tested doses (Basit et al. 2022). In contrast, Cd and Pb prolonged germination time, corroborating their known inhibitory effects on seed metabolic activities (Di Toppi, Gabbrielli 1999, Clemens 2006). The dose-dependent increase in MGT underlines the progressive delay in germination as heavy metal stress intensifies, which may affect seedling establishment and crop productivity in contaminated soils (Ertekin, Bilgen 2011, Ertekin et al. 2020, Aygün et al. 2022). Overall, these findings emphasize the importance

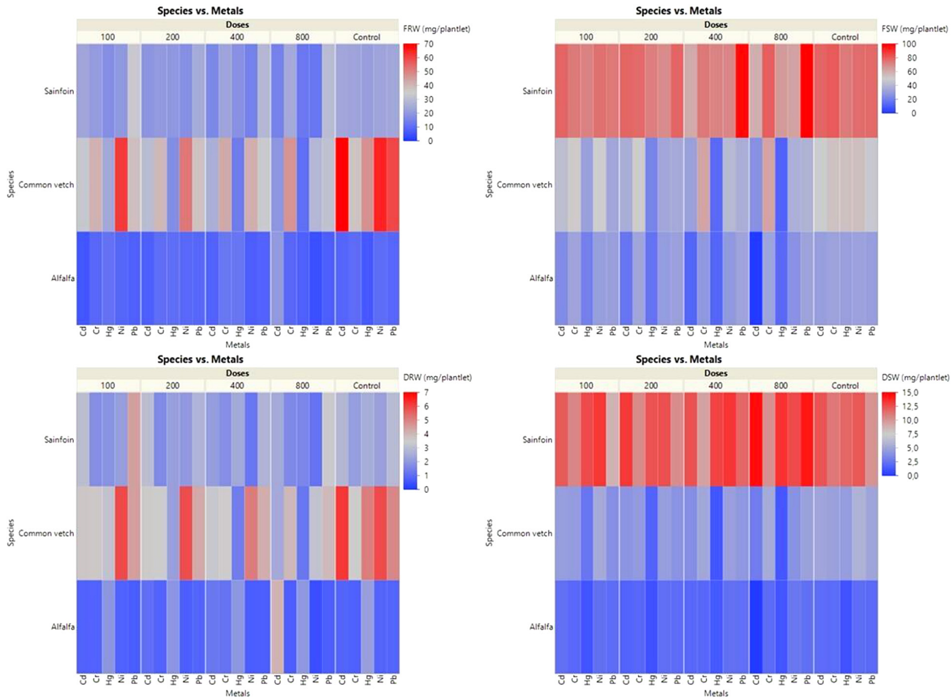

Fig. 2. Heatmaps of three-way interactions (species × metals × doses) for FRW, FSW, DRW and DSW parameters

of considering species-specific responses and metal-dose interactions in ecological risk assessments and phytoremediation strategies.

Fresh shoot weight (mg plantlet^{-1})

The analysis of variance (Table 2) showed that species (S), heavy metal type (HM), and dose (D) all significantly affected fresh shoot weight – FSW (p<0.0001), with significant interactions among these factors (S×HM, S×D, HM×D, S×HM×D, p<0.0001). Among species, sainfoin had the highest FSW (75.9 ± 1.1 mg plantlet^{-1}), significantly outperforming vetch (40.5 ± 1.4 mg plantlet^{-1}) and alfalfa (25.9 ± 0.9 mg plantlet^{-1}) (Table 3). Regarding heavy metals, chromium (Cr) and lead (Pb) treatments resulted in relatively higher FSW (55.5 ± 2.6 and 52.3 ± 3.4 mg plantlet^{-1}, respectively), while mercury (Hg) and cadmium (Cd) caused greater reductions (39.0 ± 3.3 and 42.0 ± 3.2 mg plantlet^{-1}). Increasing metal doses consistently decreased FSW, with the lowest values at 800 mg L^{-1} (43.7 ± 3.6 mg plantlet^{-1}). The heatmap (Figure 2) illustrates that sainfoin maintained relatively high shoot biomass across all metal types and doses, indicating superior tolerance. Alfalfa showed low FSW under all treatments, highlighting its sensitivity. Vetch had intermediate responses, with sharper declines under high Hg and Cd doses. These findings confirm that heavy metal stress adversely affects biomass accumulation, but species vary markedly in tolerance. Sainfoin's resilience may be attributed to its enhanced metal chelation and antioxidant mechanisms, consistent with prior studies on leguminous phytoremediators (Verbruggen et al. 2009, Goncharuk, Zagoskina 2023). Moderate toxicity observed with Cr and Pb aligns with reports that plants can partially immobilize these metals, mitigating their effects (Jawad-Hassan et al. 2020). In contrast, Hg and Cd's strong inhibition of shoot growth reflects their disruption of photosynthesis and induction of oxidative stress (DalCorso et al. 2010, Yadav 2010). The dose-dependent decline supports the literature on metal interference with nutrient uptake and cellular processes (El Rasafi et al. 2021).

Fresh root weight (mg plantlet^{-1})

ANOVA results (Table 2) showed that species (S), heavy metal type (HM), and dose (D) significantly influenced fresh root weight – FRW (p<0.0001), with all interactions also significant (p<0.0001). The Tukey's test (Table 3) indicated that common vetch had the highest FRW (38.2 ± 1.7 mg plantlet^{-1}), followed by sainfoin (21.1 ± 0.6 mg plantlet^{-1}), while alfalfa had the lowest FRW (9.1 ± 0.3 mg plantlet^{-1}). Among metals, nickel (Ni) and lead (Pb) supported higher root biomass (25.2 ± 2.6 and 25.5 ± 1.9 mg plantlet^{-1}), whereas mercury (Hg) caused the greatest inhibition (16.7 ± 1.3 mg plantlet^{-1}). Increasing metal doses progressively reduced FRW, with the lowest value at 800 mg L^{-1} (18.6 ± 1.5 mg plantlet^{-1}). The three-way interaction heatmap (Figure 2) revealed that vetch generally maintained higher FRW, especially under Pb and Ni treatments, indicating greater tolerance. Sainfoin showed moderate and variable responses, while alfalfa consistently exhibited low root biomass, reflecting greater sensitivity to heavy metal stress. These results suggest species-specific root growth adaptations to metal toxicity. Vetch's higher root biomass may relate to enhanced root exudation and metal chelation improving tolerance (Tangahu et al. 2011, Chen et al. 2017). Differences among species align with root architecture and metal uptake variability (Wu et al. 2015). Lower toxicity of Ni and Pb likely stems from detoxification and vacuolar compartmentalization (Benavides et al. 2005, Ghori et al. 2019), whereas Hg's strong inhibition corresponds to its disruption of root membranes and nutrient uptake (Seregin, Ivanov 2001, Zhu et al. 2022). The dose-dependent FRW decline highlights the detrimental impact of heavy metal accumulation on root development, potentially impairing plant establishment in contaminated soils (Hossain et al. 2012).

Dry shoot weight (mg plantlet^{-1})

ANOVA results (Table 2) showed that species (S), heavy metal type (HM), and dose (D) had significant effects on dry shoot weight – DSW (p<0.0001), with all interactions (S×HM, S×D, HM×D, S×HM×D) also being significant. The Tukey's test (Table 3) revealed sainfoin had the highest DSW (12.1 ± 0.2 mg plantlet^{-1}), followed by vetch (4.1 ± 0.1 mg plantlet^{-1}) and alfalfa (1.9 ± 0.1 mg plantlet^{-1}). Among metals, nickel (Ni) and lead (Pb) treatments supported higher DSW (6.7 ± 0.6 and 5.9 ± 0.5 mg plantlet^{-1}), while mercury (Hg) resulted in the lowest DSW (5.4 ± 0.7 mg plantlet^{-1}). Dose effects showed the highest DSW in controls (6.2 ± 0.5 mg plantlet^{-1}), with reductions at increased metal concentrations, though the highest dose (800 mg L^{-1}) caused less reduction than intermediate doses. Heatmap visualization (Figure 2) confirmed that sainfoin maintained markedly higher DSW across all metals and doses, reflecting its superior tolerance. Vetch showed intermediate DSW values, whereas alfalfa was consistently the lowest. Increasing metal doses generally reduced DSW within species, but sainfoin's shoot biomass remained relatively stable under higher stress. These results highlight that DSW is strongly affected by species identity, metal type, and concentration. Sainfoin's high DSW suggests effective physiological defenses such as antioxidant activity and metal sequestration mitigating toxicity (Hall 2002, Sharma, Dietz 2006). The higher biomass under Ni and Pb aligns with known detoxification and compartmentalization mechanisms (Lux et al. 2011, Pourrut et al. 2011), while Hg's strong inhibition corresponds to its negative impact on photosynthesis and metabolism (Rai et al. 2019). Dose-dependent biomass declines emphasize toxicity thresholds limiting growth (Kumar et al. 1995). Overall, sainfoin's biomass retention under metal stress supports its suitability for cultivation in contaminated environments.

Dry root weight (mg plantlet^{-1})

ANOVA (Table 2) revealed significant effects of species (S), heavy metal type (HM), dose (D), and their interactions on dry root weight (DRW) (p<0.0001). The Tukey's test (Table 3) showed that common vetch had the highest DRW (4.0 ± 0.1 mg plantlet^{-1}), followed by sainfoin (2.3 ± 0.1 mg plantlet^{-1}), and alfalfa with the lowest DRW (1.2 ± 0.1 mg plantlet^{-1}). Among metals, lead (Pb) and cadmium (Cd) treatments resulted in higher DRW (2.8 ± 0.2 and 2.7 ± 0.2 mg plantlet^{-1}), while chromium (Cr) and mercury (Hg) produced the lowest values (2.1–2.2 mg plantlet^{-1}). Increasing doses caused a progressive decline in DRW, with the lowest value at 800 mg L^{-1} (2.2 ± 0.2 mg plantlet^{-1}). The heatmap (Figure 2) demonstrated that vetch maintained relatively higher DRW across metals and doses, especially under Pb and Cd at high concentrations. Sainfoin showed intermediate DRW values, whereas alfalfa consistently exhibited the lowest biomass, indicating greater sensitivity. These results indicate species-specific root tolerance to heavy metals. Vetch's superior DRW likely reflects effective exclusion or detoxification mechanisms such as phytochelatin-mediated sequestration and antioxidant defenses (Clemens et al. 2013, Shahid et al. 2014). The relatively higher DRW under Pb and Cd supports partial tolerance via vacuolar compartmentalization (Cobbett, Goldsbrough 2002, Ghori et al. 2019). In contrast, Cr and Hg strongly inhibited root biomass, consistent with their disruptive effects on cellular metabolism and oxidative stress induction (Stohs, Bagchi 1995, Singh et al. 2010). Dose-dependent decreases emphasize the limits of plant defense mechanisms at high metal concentrations (Lux et al. 2011). Overall, common vetch's root biomass resilience under heavy metal stress suggests its suitability for phytoremediation of contaminated soils.

CONCLUSIONS

This study demonstrated that the type and concentration of heavy metals, as well as the plant species, significantly influence various germination and seedling growth parameters, including germination ratio, vigor, shoot and root length, and biomass accumulation. Among the tested species, sainfoin (*Onobrychis viciifolia*) and common vetch (*Vicia sativa*) exhibited greater tolerance to heavy metal stress, maintaining higher germination rates, vigor indices, and biomass production compared to alfalfa (*Medicago sativa*), which was more sensitive. In general, chromium (Cr), lead (Pb), and nickel (Ni) exerted relatively weaker inhibitory effects, whereas cadmium (Cd) and mercury (Hg) showed pronounced toxicity across all measured traits. The dose-dependent declines in growth and biomass emphasize the severity of metal toxicity at higher concentrations.

These findings highlight the potential of sainfoin and common vetch as promising candidates for phytoremediation and cultivation in moderately contaminated soils, where metal-tolerant forages could contribute to soil stabilization and ecological restoration. Furthermore, integrating such tolerant species into crop rotation or mixed cropping systems may help mitigate the risks of heavy metal accumulation in the food chain.

Future studies should focus on elucidating the physiological, biochemical, and molecular mechanisms underlying species-specific tolerance, particularly the roles of antioxidant defense, metal chelation, and ion transport pathways. A deeper understanding of these processes could support the development of breeding programs and management practices aimed at enhancing crop resilience to heavy metal stress in agricultural and ecological settings.

Author contributions

E.N.E. and M.B. – writing – original draft, visualization, validation, methodology, funding acquisition, formal analysis, data curation, conceptualization. I.E. – Software, data curation. I.E. and M.B. – writing – review & editing, validation. M.B. – project administration, investigation.

Conflicts of interest

The authors declare no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The authors received no specific funding for this work.

ACKNOWLEDGMENTS

We would like to thank the Akdeniz University Scientific Research Projects Coordination Unit (Project number: FYL-2017-2009) for financially supporting this study. This study is derived from Esra Nermin ERTEKIN's master's thesis.

REFERENCES

Alloway, B.J. (Ed.). (2012) 'Heavy metals in soils: trace metals and metalloids in soils and their bioavailability', Vol. 22, Springer Science & Business Media.

Atis, I., Atak, M., Can, E., and Mavi, K. (2011) 'Seed coat color effects on seed quality and salt tolerance of red clover (*Trifolium pratense*)', *International Journal of Agriculture & Biology*, 13(3).

Aygün, Y.Z., Atış, İ., and Ertekin, İ. (2022) 'Germination and early seedling growth of different quinoa (*Chenopodium quinoa* Willd.) genotypes under cadmium ($CdCl_2$) stress', *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi,* 27(1), 1-8, available: https://doi.org/10.37908/mkutbd.1006493

Bae, J., Benoit, D.L., and Watson, A.K. (2016) 'Effect of heavy metals on seed germination and seedling growth of common ragweed and roadside ground cover legumes', *Environmental Pollution*, 213, 112-118, available: https://doi.org/10.1016/j.envpol.2015.11.041

Basit, F., Bhat, J.A., Han, J., Guan, Y., Jan, B.L., Shakoor, A., and Alansi, S. (2022) 'Screening of rice cultivars for Cr-stress response by using the parameters of seed germination, morpho-physiological and antioxidant analysis', *Saudi Journal of Biological Sciences,* 29(5), 3918-3928, available: https://doi.org/10.1016/j.sjbs.2022.02.038

Benavides, M.P., Gallego, S.M., and Tomaro, M.L. (2005) 'Cadmium toxicity in plants', *Brazilian Journal of Plant Physiology,* 17(1), 21-34, available: https://doi.org/10.1590/S1677-04202005000100003

Can, E., Çeliktaş, N., Hatipoğlu, R., and Avcı, S. (2009) 'Breaking seed dormancy of some annual *Medicago* and *Trifolium* species by different treatments', *Turkish Journal of Field Crops*, 14(2), 72-78.

Chen, Y.T., Wang, Y., and Yeh, K.C. (2017) 'Role of root exudates in metal acquisition and tolerance', *Current Opinion in Plant Biology*, 39, 66-72.

Clemens, S., (2006) 'Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants', *Biochimie*, 88(11), 1707-1719, available: https://doi.org/10.1016/j.biochi.2006.07.003

Clemens, S., Aarts, M.G.M., Thomine, S., and Verbruggen, N. (2013) 'Plant science: The key to preventing slow cadmium poisoning', *Trends in Plant Science,* 18(2), 92-99, available: https://doi.org/10.1016/j.tplants.2012.08.004

Cobbett, C., and Goldsbrough, P. (2002) 'Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis', *Annual Review of Plant Biology,* 53(1), 159-182, available: https://doi.org/10.1146/annurev.arplant.53.100301.135154

DalCorso, G., Farinati, S., and Furini, A. (2010) 'Regulatory networks of cadmium stress in plants', *Plant Signaling & Behavior,* 5(6), 663-667, available: https://doi.org/10.4161/psb.5.6.11503

Di Toppi, S.L., and Gabbrielli, R. (1999) 'Response to cadmium in higher plants', *Environmental and Experimental Botany*, 41(2), 105-130, available: https://doi.org/10.1016/S0098-8472(98)00073-6

El Rasafi, T., Bouda, S., Hamdali, H. and Haddioui, A. (2021) 'Seed germination and early seedling growth of fenugreek (*Trigonella foenum-gracium* L.) under Cu, Ni and As stress', *Acta Ecologica Sinica,* 41(3), 223-227.

Ertekin, E.N., and Bilgen, M. (2021) 'Effects of some heavy metals on germination and early seedling growth of maize (*Zea mays* L.)', *Biological Diversity and Conservation,* 14(2), 198-207, available: https://doi.org/10.46309/biodicon.2021.889901

Ertekin, E.N., Ertekin, İ., and Bilgen, M. (2020) 'Effects of some heavy metals on germination and seedling growth of sorghum', *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi,* 23(6), 1608-1615, available: https://doi.org/10.18016/ksutarimdoga.v23i54846.722592

Ertekin, İ., Yılmaz, Ş., and Can, E. (2022). 'Bazı yumak (*Festuca* spp.) türlerinin çimlenme ve fide aşamasında tuz stresine tepkilerinin belirlenmesi' *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*, *27*(2), 266-271.

Ertekin, İ., Yılmaz, Ş., Atak, M., & Can, E. (2018) 'Effects of different salt concentrations on the germination properties of Hungarian vetch (*Vicia pannonica* Crantz.) cultivars' *Türk Tarım ve Doğa Bilimleri Dergisi*, *5*(2), 175-179.

Ertekin, İ., Yılmaz, Ş., Atak, M., Can, E., and Çeliktaş, N. (2017) 'Tuz stresinin bazı yaygın fiğ (*Vicia sativa* L.) çeşitlerinin çimlenmesi üzerine etkileri', *Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi*, 22(2), 10-18.

Ghori, N.H., Ghori, T., Hayat, M.Q., Imadi, S.R., Gul, A., Altay, V., and Ozturk, M. (2019) 'Heavy metal stress and responses in plants', International Journal of Environmental Science and Technology, 16(3), 1807-1828, available: https://doi.org/10.1007/s13762-019-02215-8

Ghosh, M., and Singh, S.P. (2005) 'A review on phytoremediation of heavy metals and utilization of it's by products', *Asian Journal of Energy and Environment,* 6(4), 18.

Goncharuk, E.A., and Zagoskina, N.V. (2023) 'Heavy metals, their phytotoxicity, and the role of phenolic antioxidants in plant stress responses with focus on cadmium', *Molecules,* 28(9), 3921, available: https://doi.org/10.3390/molecules28093921

Gupta, D.K., Huang, H.G., Yang, X.E., Razafindrabe, B.H.N., and Inouhe, M. (2010) 'The detoxification of lead in *Sedum alfredii* H. is not related to phytochelatins but the glutathione', *Journal of Hazardous Materials*, *177*(1-3), 437-444, available: https://doi.org/10.1016/j.jhazmat.2009.12.052

Hall, J.Á. (2002) 'Cellular mechanisms for heavy metal detoxification and tolerance', *Journal of Experimental Botany,* 53(366), 1-11, available: https://doi.org/10.1093/jxb/53.366.1

He, J., Xu, Z., Zhang, Z., and Chen, W. (2015) 'Toxic effects of mercury on plant growth, photosynthesis, and antioxidant enzyme activity in *Brassica chinensis* L.', *Ecotoxicology and Environmental Safety,* 113, 273-279. https://doi.org/10.1016/j.ecoenv.2014.12.011

Hossain, M.A., Piyatida, P., da Silva, J.A.T., and Fujita, M. (2012) 'Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation', *Journal of Botany,* 1, 872875, available: https://doi.org/10.1155/2012/872875

Jawad-Hassan, M., Ali Raza, M., Ur Rehman, S., Ansar, M., Gitari, H., Khan, I., Wajid, M., Ahmed, M., Shah, G.A., Peng, Y., and Li, Z. (2020) 'Effect of cadmium toxicity on growth, oxidative damage, antioxidant defense system and cadmium accumulation in two sorghum cultivars', *Plants,* 9(11), 1575, available: https://doi.org/10.3390/plants9111575

Kumar, P.N., Dushenkov, V., Motto, H., and Raskin, I. (1995) 'Phytoextraction: the use of plants to remove heavy metals from soils', *Environmental Science & Technology,* 29(5), 1232-1238.

Liu, W., Zhou, Q., An, J., Sun, Y., and Liu, R. (2010) 'Variations in cadmium accumulation among Chinese cabbage cultivars and screening for Cd-safe cultivars', *Journal of Hazardous Materials*, *173*(1-3), 737-743. https://doi.org/10.1016/j.jhazmat.2009.08.147

Lux, A., Martinka, M., Vaculík, M., and White, P.J. (2011) 'Root responses to cadmium in the rhizosphere: A review', *Journal of Experimental Botany,* 62(1), 21-37, available: https://doi.org/10.1093/jxb/erq296

Nagajyoti, P.C., Lee, K.D., and Sreekanth, T.V.M. (2010) 'Heavy metals, occurrence and toxicity for plants: a review', *Environmental Chemistry Letters,* 8(3), 199-216, available: https://doi.org/10.1007/s10311-010-0297-8

Pourrut, B., Shahid, M., Dumat, C., Winterton, P., and Pinelli, E. (2011) 'Lead uptake, toxicity, and detoxification in plants', *Reviews of Environmental Contamination and Toxicology,* 213, 113-136, available: https://doi.org/10.1007/978-1-4419-9863-7_5

Rai, P.K., Lee, S.S., Zhang, M., Tsang, Y.F., and Kim, K.H. (2019) 'Heavy metals in food crops: Health risks, fate, mechanisms, and management', *Environment International,* 125, 365-385, available: https://doi.org/10.1016/j.envint.2019.01.067

Seregin, I. V., and Ivanov, V.B. (2001) 'Physiological aspects of cadmium and lead toxic effects on higher plants', *Russian Journal of Plant Physiology,* 48(4), 523-544, available: https://doi.org/10.1023/A:1012458421001

Shahid, M., Dumat, C., Khalid, S., Schreck, E., Xiong, T., and Antunes, P.M. (2014) 'Heavy-metal-induced reactive oxygen species: Phytotoxicity and physicochemical changes in plants', *Reviews of Environmental Contamination and Toxicology,* 232, 1-44, available: https://doi.org/10.1007/978-3-319-58811-3_1

Sharma, P., and Dietz, K.J. (2006) 'The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress', *Journal of Experimental Botany,* 57(4), 711-726, available: https://doi.org/10.1093/jxb/erj089

Sharma, P., and Dubey, R.S. (2005) 'Lead toxicity in plants', *Brazilian Journal of Plant Physiology,* 17(1), 35-52. https://doi.org/10.1590/S1677-04202005000100004

Seregin, I.V., and Kozhevnikova, A.D. (2008) 'Roles of root and shoot tissues in transport and accumulation of cadmium, lead, nickel, and strontium', *Russian Journal of Plant Physiology*, 55(1), 1-22, available: https://doi.org/10.1134/S1021443708010019

Singh, R., Tripathi, R.D., Dwivedi, S., Kumar, A., Trivedi, P.K., and Chakrabarty, D. (2010) 'Lead bioaccumulation potential of an aquatic macrophyte *Najas indica* are related to antioxidant system', *Bioresource Technology,* 205, 99-107, available: https://doi.org/10.1016/j.biortech.2009.12.031

Singh, S., Parihar, P., Singh, R., Singh, V. P., and Prasad, S. M. (2016) 'Heavy metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics, and ionomics', *Frontiers in Plant Science,* 6, 1143, available: https://doi.org/10.3389/fpls.2015.01143

Stohs, S.J., and Bagchi, D. (1995) 'Oxidative mechanisms in the toxicity of metal ions', *Free Radical Biology and Medicine,* 18(2), 321-336, available: https://doi.org/10.1016/0891-5849(94)00159-0

Tangahu, B.V., Sheikh Abdullah, S.R., Basri, H., Idris, M., Anuar, N., and Mukhlisin, M. (2011) 'A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation', *International Journal of Chemical Engineering,* 2011(1), 939161, available: https://doi.org/10.1155/2011/939161

Timm, S., Woitschach, F., Heise, C., Hagemann, M., & Bauwe, H. (2019) 'Faster removal of 2-phosphoglycolate through photorespiration improves abiotic stress tolerance of Arabidopsis' *Plants*, 8(12), 563, available: https://doi.org/10.3390/plants8120563

Verbruggen, N., Hermans, C., and Schat, H. (2009) 'Mechanisms to cope with arsenic or cadmium excess in plants', *Current Opinion in Plant Biology,* 12(3), 364-372, available: https://doi.org/10.1016/j.pbi.2009.04.005

Wu, F., Fu, L., Zhang, G., and Huang, H. (2015) 'Comparative study of heavy metal accumulation and tolerance in three legume species', *Ecotoxicology,* 24(6), 1231-1241, available: https://doi.org/10.1007/s10646-015-1483-7

Yadav, S.K. (2010) 'Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants', *South African Journal of Botany,* 76(2), 167-179, available: https://doi.org/10.1016/j.sajb.2009.10.007

Zhao, F.J., Hamon, R.E., Lombi, E., McLaughlin, M.J., and McGrath, S.P. (2002) 'Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*', *Journal of Experimental Botany,* 53(368), 535-543, available: https://doi.org/10.1093/jexbot/53.368.535

Zhu, Y., Dong, Y., Zhu, N., and Jin, H. (2022) 'Foliar application of biosynthetic nano-selenium alleviates the toxicity of Cd, Pb, and Hg in *Brassica chinensis* by inhibiting heavy metal adsorption and improving antioxidant system in plant', *Ecotoxicology and Environmental Safety,* 240, 113681, available: https://doi.org/10.1016/j.ecoenv.2022.113681